The National Honey Bee Disease and Pest Survey:
2009-2010 Pilot Study Summary Report

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Executive summary

This pilot study was conducted to 1) validate and trouble shoot the sample collection process we proposed to use for a national survey effort, 2) assess the infrastructures related to shipping, storing and analyzing the specimens, and 3) gather baseline data for a broader survey of honey bee pests and pathogens that was initiated in 2010. The participating states were California, Florida, and Hawaii and a total of 87 samples were collected.

We found that our collection protocol worked well, and found that shipping lives bees is a good and viable alternative to collecting and shipping bees on dry ice; however, the rate of surviving bees decreases dramatically with transit times longer than 5 days.

In all, samples from 13 different organisms with known associations with managed honey bees were examined. We found three viruses, Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV) and Kashmir Bee Virus (KBV) in all surveyed states. Chronic Bee Paralysis Virus (CBPV) and Israeli Acute Paralysis Virus (IAPV) were found in both California and Florida, but not in Hawaii. Slow Paralysis Virus (SPV) was not found in any samples. While *N. ceranae* was ubiquitous in all samples, *N. apis* was notably absent, none being detected in any samples. Tracheal mites and Tropilaelaps mites were also not found in any samples. Varroa mites were found in all states, and were found particularly abundantly in some Hawaii samples.

This survey was not designed to be comprehensive representation of the country, and the results should not be interpreted to mean the absence of certain pathogens in the US or in any one particular state.

Introduction

A pilot survey of honey bee pests and diseases was funded in 2009 by the USDA Animal Plant Health Inspection Service (APHIS) and was concluded in 2010. This survey was conducted in an attempt to document which bee diseases and parasites of honey bees are currently present in the U.S., and to examine all samples for Tropilaelaps, a parasitic mite not thought to be in the U.S. This pilot survey was initiated to validate and trouble shoot the sample collection process,

assess the infrastructures related to shipping, storing and analyzing the specimens, and to gather baseline data for a broader survey of honey bee pests and pathogens that was initiated in 2010. The three states surveyed by this limited effort were California, Hawaii and Florida and a total of 87 apiaries, representing 696 colonies were sampled.

California, Florida and Hawaii were chosen because they represent high-risk areas that have many potential ports of entry, long growing seasons, and diverse agricultural crops. Twenty-five samples were collected from different voluntary apiaries throughout Florida, and fourteen samples from Hawaii. Forty eight samples were collected from California, twenty seven from hives originating in that state and twenty one from migratory beekeepers who were in California under pollination contracts or other reasons.

Coordination of this survey is in collaboration with USDA Agricultural Research Service (ARS) Bee Research Lab (BRL) in Beltsville, MD, Pennsylvania State University (PSU), the Florida Department of Agriculture and Consumer Services (FDACS) and USDA APHIS.

Survey Description

Live samples taken in the field were sent to USDA BRL and immediately frozen at -80^{0}C upon arrival. The frozen samples were held until molecular analysis was conducted. Molecular testing of the samples was focused on identifying the following viruses, and pathogens:

1. Acute Bee Paralysis Virus (ABPV)
2. Chronic Bee Paralysis Virus (CBPV)
3. Deformed Wing Virus (DWV)
4. Israeli Acute Paralysis Virus (IAPV)
5. Kashmir Bee Virus (KBV)
6. Slow Paralysis Virus (SPV)
7. Trypanosome sp.
8. *Nosema ceranae*
9. *Nosema apis*

The samples taken at the apiaries and preserved in alcohol were later inspected using microscopic analysis at Pennsylvania State University and USDA BRL to:

1. Quantify Nosema spores
2. Quantify Tracheal Mites loads
3. Detect Tropilaelaps Mites
4. Quantify Varroa Mite loads

Beekeepers participating in this survey were provided with a summary report on the average apiary level Nosema, tracheal mites, and Varroa loads in addition to the presence or absence of

Tropilaelaps. This report was also furnished to each state-level apiary specialist. A separate report that presented the results from the molecular analysis of the sampled bees was distributed to the participating beekeepers and state-level apiary specialists. This report provided the participant with a positive or negative result for the six bee viruses targeted, the two Nosema species targeted, and the presence or absence of Trypanosome in the sampled apiary.

Part of the survey included a visual inspection of the hives before sampling; therefore, the presence of the following symptoms, pests and brood diseases was also recorded, but not analyzed, at the apiaries for each sample taken:

1. American Foul Brood
2. Black Shiny Bees
3. Chalkbrood
4. Deformed Wing Virus
5. European Foul Brood
6. Parasitic Mite Syndrome
7. Sac Brood
8. Small Hive Beetle Adults/Larvae
9. Wax Moth Adults/Larvae

Evaluation of sampling protocol

Live bees were shipped via the U.S. Postal service from each apiary to Beltsville, MD for molecular testing. In each live bee 'kit' was a petri dish that contained both a small amount of water and some hard "queen" candy for food for the bees. This kit contained approximately 12000 live adult bees at sampling time. The percentage of bees lost in transit was directly affected by the length of time samples were in transit (Figure 1). There was a noticeable decline in the percentage of live bees surviving in sampling boxes when they took 5 days or longer to arrive. It is not known whether this was due to temperatures experienced during shipping or a lack of food or water or a combination of all three variables.

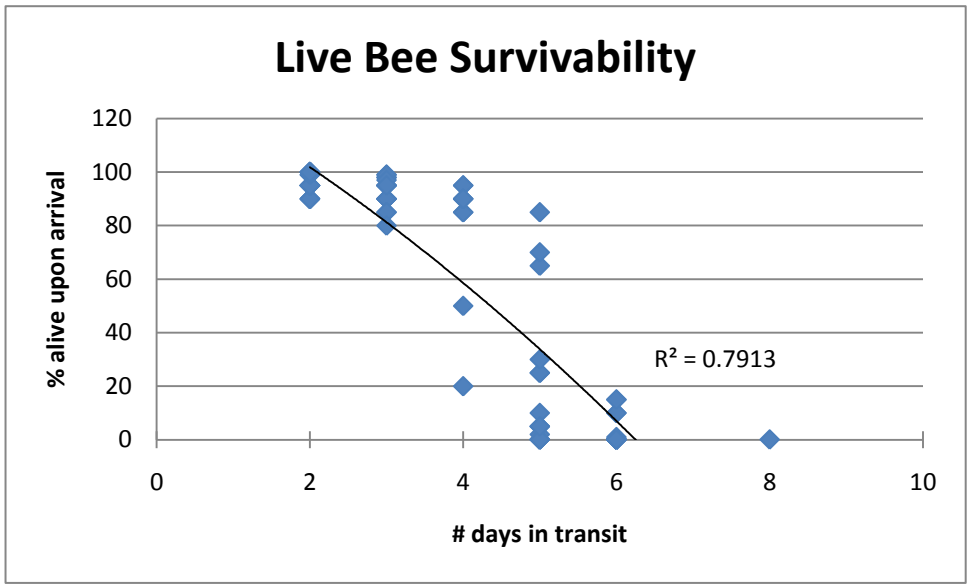

Figure 1: Live Bee Survivability Plot (R=.89)

The geographic distribution of the samples for each state is given in Figures 2-4. The numerical markers on these maps indicate the number of apiaries assessed in that general location from July 2009 through June 2010. Samples from the Hawaiian Islands included Kauai, Oahu and the island of Hawaii (the Big Island).

Figure 2: Geographical Distribution of California Pilot Samples

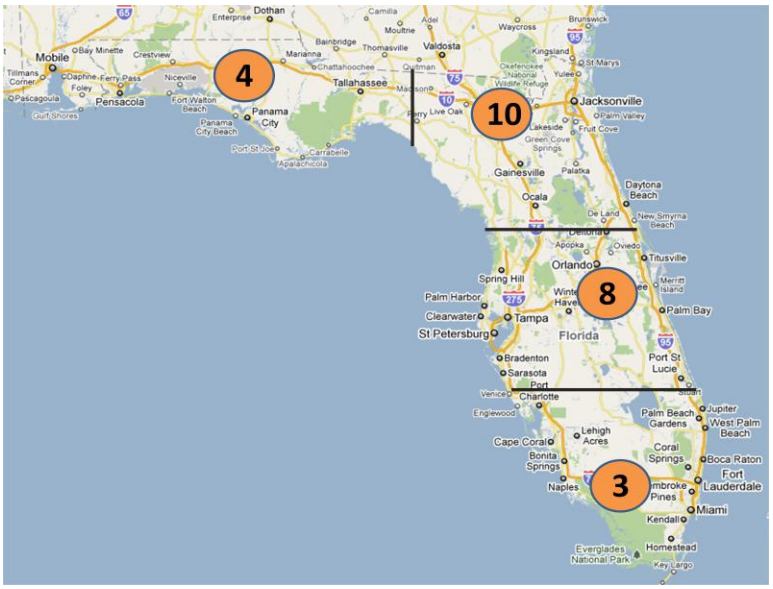

Figure 3: Geographical Distribution of Florida Pilot Samples

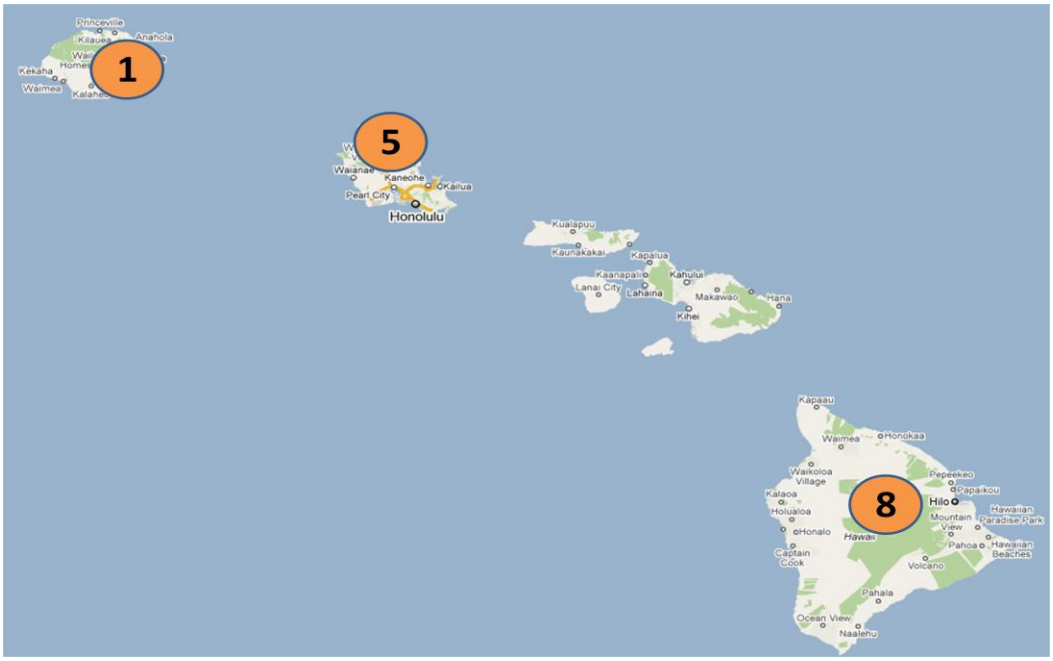

Figure 4: Geographical Distribution of Hawaii Pilot Samples

Results

The results of molecular analysis are given in Figure 5. This graph shows the prevalence of pathogen detection in aggregate apiary level samples taken from all states. Neither Slow Paralysis Virus (SPV) nor *Nosema apis* were found in any samples.

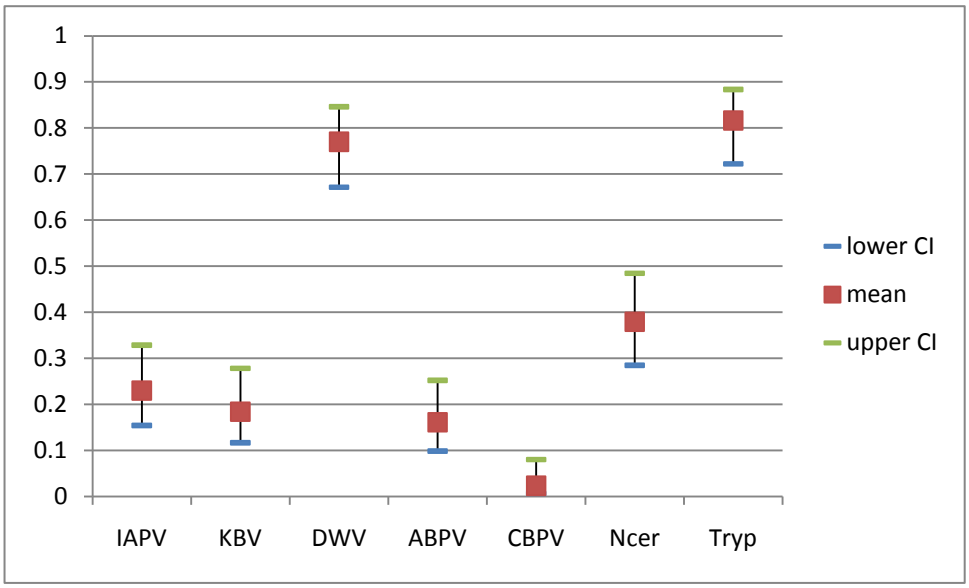

Figure 5: Prevalence of viruses and pathogens in sampled apiaries. The point estimate and (95% Confidence intervals are reported)

The average Nosema load per bee (in millions of spores) and the average Varroa load per 100 bees are portrayed in Figure 6. As *Nosema apis* was not observed by molecular analysis in any sample, it can be assumed that all the Nosema identified by microscopic identification was *Nosema ceranae*. Prevalence of Varroa in samples ranged from no Varroa detected to almost 19 mites per 100 bees. N. ceranae levels ranged from none detected to over 4 million spores per bee. Tracheal and Tropilaelaps mites were not detected in any sample.

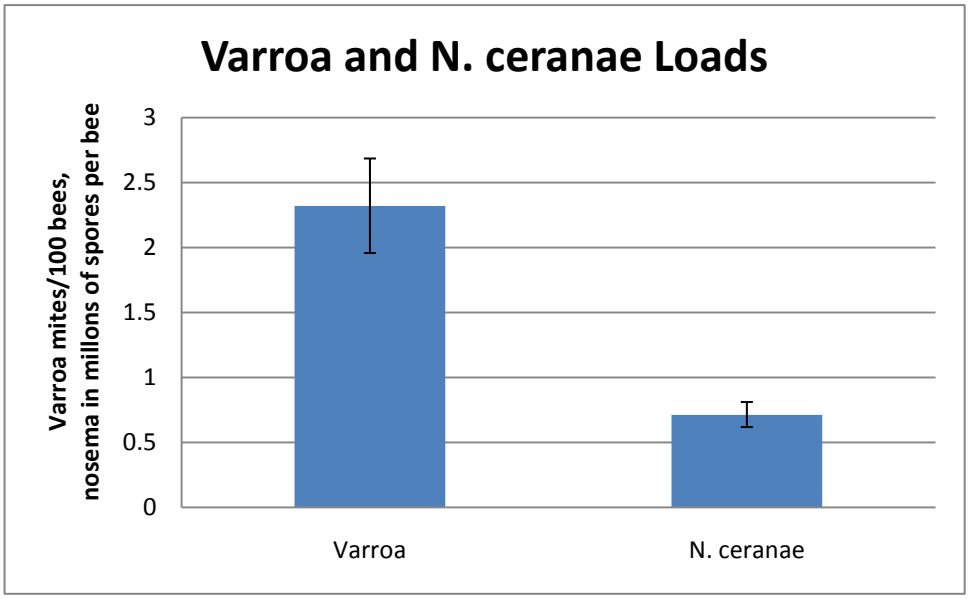

Figure 6: Prevalence of Varroa and N. ceranae in sampled apiaries. (Standard Error bars are reported)

Conclusions

The sample protocol developed worked well and the shipping and storage methods were sufficiently robust to justify the initiation of a national effort. The sample size and sampling effort were not robust enough to make any categorical statements about the absence of parasites in the US. So, while no Tropilaelaps mites were found in these efforts, neither were honey bee tracheal mites nor *Nosema apis*, both of which are known to be present.